THE ASSOCIATION OF SERUM LONG-CHAIN OMEGA-3 POLYUNSATURATED FATTY ACIDS WITH SERUM RESISTIN

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Resistin concentration and long chain omega-3 PUFA (n-3 PUFA) are both integrated with the risk of cardiovascular disease (CVD) and diabetes. However, there is little prior information about the association between the serum n-3 PUFA and serum resistin concentration in healthy population.

A total of 892 men free of CVD and type 2 diabetes from prospective, population-based Kuopio Ischemic Heart Disease Risk Factor study (KIHD) at baseline in 1984–1989, were studied. Serum esterified and non-esterified fatty acids were determined in one gas chromatographic run without pre-separation. Serum long chain n-3 PUFA was set as an exposure. Dietary intake of nutrients was assessed quantitatively with a 4-day food recording at the KIHD baseline examinations. Intake of nutrients was calculated by use of Nutrica version 2.5 software. Serum resistin concentration was measured using ELISA kit. The univariate associations between the serum n-3 PUFAs concentration and demographic, lifestyle and clinical characteristics at baseline were assessed by means and linear regression for continuous variables and chi²-test for categorical variables. We used linear regression models to determine the association between serum n-3 PUFAs and serum resistin level and analysis of covariates to estimate the average resistin level in quartiles of the serum long-chain n-3 PUFA.

The mean age was 61.7 years (s.d. 6.4). The mean serum concentration as a percentage of all serum fatty acids was 2.78 % for docosahexaenoic acid (DHA) (s.d. 0.90), which was highest serum concentration among all long chain n-3 PUFAs, followed by eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) which were 1.66% (s.d. 0.92) and 0.78% (s.d. 0.16) respectively. The mean concentration of serum resistin was 1.92 ng/ml (s.d. 0.67 ng/ml). Serum resistin was inversely associated with total n-3 PUFA and with individual long chain n-3 PUFA, DPA and DHA after age, gender and examination year adjustment. However, after further adjustments, only the association between the serum DHA and serum resistin concentration remained significant. No significant associations were observed between the EPA and serum resistin concentration in both models.

We observed that serum long chain n-3 PUFA and especially DHA, an objective biomarker of fish or fish oil consumption, were inversely associated with serum resistin concentration. As resistin is known as a potential marker for increased risk of obesity, inflammation, diabetes and heart diseases, the beneficial effect of serum long chain n-3 PUFA on these diseases can be partially explained by their impact on serum resistin concentration.
Abbreviations

ALA: alpha linolenic acid
AMI: acute myocardial infarction
BMI: body mass index
CAD: coronary artery disease
CRP: C-reactive protein
CHD: coronary heart disease
CVD: cardiovascular disease
DA: Dalton molecular scale
DHA: docosahexaenoic acid
DPA: docosapentaenoic acid
EPA: eicosapentaenoic acid
HC: hip circumference
HDL: high-density lipoprotein
HR: heart rate
KIHD: Kuopio Ischaemic Heart Disease Risk Factor study
IL-6: interleukin-6
LDL: low-density lipoprotein
MUFA: monounsaturated fatty acid
N-3 PUFA: long-chain n-3 polyunsaturated fatty acids
PCI: percutaneous coronary intervention
PBMC: peripheral blood mononuclear cells

PPAR\(\gamma\): peroxisome proliferator-activated receptor gamma

POS: polycystic ovary syndrome

RELM: resistin-like molecule

SAFA: saturated fatty acid

T2D: type 2 diabetes

TG: triglyceride

TNF: tumor necrosis factor

WC: waist circumference

WHR: waist to hip ratio
"Science is organized knowledge. Wisdom is organized life”

Immanuel Kant

To my family Mr. N. Tajik, Mrs. A. Cheraghi and Mr. B. Tajik

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INTRODUCTION

One of the biggest challenges in the twenty-first century is the increasing burden of chronic diseases like cardiovascular disease (CVD) and diabetes, which are responsible for the large portion of mortality and disability in the whole world (World Health Organization). For this reason, ever increasing importance of recognizing the factors which are associated with these chronic diseases becomes undeniable. Diet and nutritional factors have significant role in prevention of CVD and diabetes, and also take part in the CVD and diabetes treatments. Amid nutritional factors, long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) seem to ameliorate obesity and may play a crucial role in the lower incidence of CVD (Mozaffarian & Wu 2011). Moreover, one of the recently discovered factor, which has been associated with the risk of diabetes and CVD is resistin (McTernan et al. 2002, Reilly et al. 2005, Burnett et al. 2006a). N-3 PUFA seems to alter resistin concentration; however, the association between intake of n-3 PUFA and resistin concentration has not been well evaluated.

There is little prior information about the association between the serum n-3 PUFA and serum resistin concentration in healthy population. However, over the past years, relevant studies have been conducted among animals and findings about the association between the n-3 PUFA and serum resistin concentration are inconsistent (Haugen et al. 2005, Ye Wang 2014). In the population-based studies, the association between the n-3 PUFA and resistin concentration/expression were carried out among people with coronary heart disease (CHD) and acute myocardial infraction (AMI) and results are highly contrasting (Mizia-Stec et al. 2011, Mostowik et al. 2013, Oner & Muderris 2013). Primary objective of the current study was to evaluate the association between the serum n-3 PUFA and serum resistin concentration in healthy population.
2 LITERATURE REVIEW

2.1 Resistin

Adipose tissue is an active metabolic and endocrine organ, which secretes numerous substances including hormones, growth factors, cytokines, chemokines and adipokines (Morrison & Farmer 2000). Adipokines are bioactive substances, which include adiponectin, leptin, ghrelin and resistin (Deng & Scherer 2010). Adipokines provide a link between the obesity and inflammation disorders and they may influence on the weight regulation by affecting appetite, energy expenditure, and glucose and lipid homeostasis (Mora & Pessin 2002), though the data are not consistent.

Adiponectin is a collagen-like protein that is mainly secreted by the adipocytes and it is known as an anti-inflammatory and an insulin-sensitizer factor. Also it could suppress the risk of atherosclerosis and liver diseases (Kadowaki et al. 2006). Leptin is another adipokines, which is secreted from the white adipocytes and it may affect the whole-body energy balance in rodents and humans (Houseknecht et al. 1998).

Among adipokines, role of resistin in body was the interest in previous studies. Resistin is a cysteine-rich adipose-derived peptide hormone and is a member of the resistin-like molecule (RELM) family with 12.5k Dalton Molecular scale (DA) molecular weight (Steppan et al. 2001). It includes mature sequence of 108 amino acid (Steppan et al. 2001). Resistin originally named for its resistance to insulin (Steppan et al. 2001). In mice, resistin expression occurs predominantly in the adipocytes (Steppan et al. 2001, Nogueiras et al. 2003, Milan et al. 2002). In contrast, it has been suggested that significant level of resistin expression predominantly occurs in the macrophages (mainly), peripheral blood mononuclear cells (PBMCs), bone marrow, spleen and mature adipocytes (Patel et al. 2003, Janke et al. 2002, Fain et al. 2003, Degawa-Yamauchi et al. 2003).

Resistin is known as a pro-inflammatory cytokine that seems to be a link between the obesity and type II diabetes (T2D) (Holcomb et al. 2000, Steppan et al. 2001). It has been reported that resistin concentration is directly associated with the risk of breast cancer (Wei-kai et al. 2007, Hou et al. 2007), myocardial infarction (Weikert et al. 2008), colon cancer (Salageanu et al. 2010, Gonullu et al. 2010, Nakajima et al. 2010), and rheumatoid arthritis (Senolt et al. 2007, Otero et al. 2006).
2.2 Polyunsaturated fatty acids

PUFA are fatty acids, which structurally have double bonds. PUFA are divided into two categories: n-3 PUFA (omega-3) and n-6 PUFA (omega-6). N-3 PUFA are fatty acids, which have a double bond at the carbon atom number 3, while in n-6 PUFA this double bond sits at carbon atom number 6 (Russo 2009, Ruxton et al. 2004).

Individual n-3 PUFAs are: 1- Alpha linolenic acid (ALA) (18:3n-3) is an essential fatty acid, which cannot be synthesized in human body and is obtained from the plants sources such as canola, rapeseed oils, linseed oils and nuts (Burdge & Calder 2005, Barceló-Coblijn & Murphy 2009). It has been shown that ALA may improve insulin sensitivity by decreasing lipid accumulation in skeletal muscle and liver (Muramatsu et al. 2010). Moreover, some studies reported that higher intake of ALA is inversely associated with the risk of CVD (Mozaffarian 2005).

2- Eicosapentaenoic acid (EPA) (20:5n-3) and decosahexaenoic acid (DHA) (22:6n-3) are derived mainly from seafood such as anchovy, bluefish, herring, mackerel, mullet, sardines, salmon, sturgeon, tuna and other marine food sources and fish oils (Saldeen & Saldeen 2006). Little part of plasma ALA is destined for the synthesis of EPA and DHA in healthy individuals due to various factors (0.2% and 8% - 0% to 4% respectively). For example, it has been indicated that higher intake of ALA leads to increased conversion of ALA to EPA, and EPA to DHA (Burdge & Calder 2005, Barceló-Coblijn & Murphy 2009). Moreover, this conversion is higher in women as compared to men. Since this conversion cannot meet the EPA demand, the majority of DHA and EPA are obtained from diet (Burdge & Calder 2005, Barceló-Coblijn & Murphy 2009).

3- Docosapentaenoic acid (DPA) is an EPA metabolite. Since fish contains limited amount of DPA (Kaur et al. 2011) and the correlation between the fish consumption and circulating DPA is weaker than DHA and EPA (von Schacky & Weber 1985, Arterburn et al. 2006), the major source of DPA is based on internal metabolism rather than directly from diet.

2.2.1 N-3 PUFA recommendation

According to the European Food Safety Authority dietary recommendations, 250-500mg/day EPA and DHA may decrease the risk of CVD in healthy adult population (Djuricic et al. 2014). Moreover, International Society for the Study of Fatty Acids and Lipids recommend at least 500 mg/day (EPA+DHA) for primary prevention of CHD (Cunnane et al. 2004). American Heart Association (AHA) recommended intake of 1 g EPA+DHA per day from fish oil as a secondary
prevention for people with CHD background (American Heart Association Nutrition Committee et al. 2006).

2.3 Possible association between the n-3 PUFAs and resistin level

Resistin concentration and n-3 PUFA are both integrated with the health issues such as heart disease, insulin resistance and diabetes, obesity and inflammation, which suggest that there may be an association between them.

2.3.1 Heart diseases

It has been reported that resistin can play a pathogenic role in development of atherosclerosis and coronary artery diseases (CAD) (Jamaluddin et al. 2012, Lee & Kim 2012, Lim et al. 2008, Burnett et al. 2006, Tuttolomondo et al. 2010). Resistin concentration can be a risk marker for ischemic heart disease in the generally healthy population (Cabrera de León et al. 2014) since resistin concentration is directly associated with the coronary calcium score, which is one of the marker of atherosclerosis (Reilly et al. 2005). Resistin concentration is also directly associated with the incidence of atherosclerosis because of its role in inflammation process (Ding et al. 2011). It has been shown that resistin concentration was significantly higher in 220 patients coronary acute syndrome as compared to healthy participants (Wang et al. 2009). This finding was in agreement among patients with CAD (Ohmori et al. 2005).

However, some studies did not found any association between resistin concentration and risk of CVD. A study conducted among 547 consecutive patients (age 63 ± 10 years) undergoing coronary angiography reported that there is no associations between resistin concentration with the presence of coronary stenoses and also with incidence of future vascular events (Hoefle et al. 2007). This finding was supported with another study among 1162 subjects with and without angiographic coronary artery disease (Pilz et al. 2007).

The association between higher resistin concentration and CVD may be partially explained by the association between resistin concentration and endothelial function. Endothelial function is strongly correlated with the endothelial nitric oxide synthase system in human coronary artery endothelial cells (Murohara et al. 1998, Palmer et al. 1987, Zeng & Quon 1996, Moore et al. 2011). It has been shown that resistin concentration is inversely associated with the expression of endothelial nitric oxide synthase (Chen et al. 2010, Chen et al. 2002, Verma et al. 2003, Kawanami et al. 2004).
It has been indicated that n-3 PUFA play an important role in the lower incidence of CVD by its impacts on cardiovascular risk factors (Mozaffarian & Wu 2011). N-3 PUFA consumption may lead to reduction in plasma triglyceride (TG) (Harris & Bulchandani 2006), decreased resting heart rate (HR) and HR respond to exercise (Mozaffarian & Wu 2011, Peoples et al. 2008), lower systolic and diastolic blood pressure (Geleijnse et al. 2002) and improvement in cardiac filling and myocardial efficiency (Pepe & McLennan 2002). Moreover, it has been pointed out that n-3 PUFA may have an anti-thrombotic effects (Wang et al. 2004).

According to the recent prospective, population-based Kuopio Ischemic Heart Disease Risk Factor study (KIHD), among 1981 men aged 42–60 years and free of CVD, dietary n-3 PUFA intake was associated with the lower risk of fatal, but not with non-fatal CHD (Virtanen et al. 2012). Some factors may have effects on the association between the fish intake and risk of CHD. For example, it has been suggested that inverse association between fish intake and risk of CHD was stronger in women than men (Järvinen et al. 2006). Mercury content, which is known as an environmental contaminant, is another factor, which can attenuate the association between fish intake and risk of CHD (Rissanen et al. 2000, Virtanen et al. 2005).

2.3.2 Insulin resistance and diabetes

Insulin resistance is a condition, which the body cells do not respond properly to the insulin. In this condition, the insulin that is produced in the body is more than normal and it cannot function sufficiently in skeletal muscle, liver and adipocytes (Caro 1991, Shulman 2000). It has been suggested that insulin resistance is linked to the obesity and it often leads to T2D and CVD (Bo et al. 2005, Balkau & Eschwege 1999).


Resistin can be a potential risk factor in central obesity, which may lead to T2D (McTernan et al. 2002). It has been shown that resistin concentration was directly associated with the insulin resistance in population-based studies (Silha et al. 2003). In addition, the level of resistin increased in diabetic participants as compared to non-diabetic participants (McTernan et al. 2002).
to (Gharibeh et al. 2010) findings among 125 participants suffering from T2D, serum resistin concentration in obese diabetic participants was higher than non-diabetics. Resistin mRNA expression seems to be higher in diabetic women as compared to the non-diabetic women (Tsiotra et al. 2008). It has been also indicated that resistin concentration is higher in patients with gestational diabetes that pregnant women with normal glucose tolerance (Kuzmicki et al. 2009).

In contrast, some studies did not show any association between the serum resistin concentration and risk of T2D (Pfutzner et al. 2003, Stejskal et al. 2003, Lee et al. 2003, Levy et al. 2002). Regarding to the cross-sectional study in 123 middle-aged and 120 healthy young subjects, resistin concentration was not correlated with BMI, WHR, fat mass and insulin resistance. Also resistin concentrations were non different in diabetics and non-diabetics (Lee et al. 2003). Moreover, it has been demonstrated that resistin gene expression is not associated with insulin resistance determinants in human abdominal adipocyte (Janke et al. 2002).

The evidences regarding to an association between the n-3 PUFA and diabetes remained unclear. It has been suggested that n-3 PUFA is associated with the risk of diabetes since it may have association with diabetes risk factors such as hypertension, inflammation and dyslipidemia and the translocation of glucose transporters. Recently in KIHD, serum n-3 PUFA was inversely associated with the risk of T2D among 2212 middle-aged and older men (Virtanen et al. 2014).

However, in two meta analyses among 40184 individuals and 25670 cases of incident diabetes, and among 527441 participants and 24082 diabetes cases, they could not find a statistically significant association between EPA and DHA intake with risk of diabetes (Wu et al. 2012, Wallin et al. 2012). According to another meta-analysis, which was conducted among 24509 T2D patients and 545275 participants, n-3 PUFA was inversely associated with the risk T2D among Asians population. In contrast, a direct association has been found between the n-3 PUFA consumption and risk of T2 diabetes among western study population (Zheng et al. 2012).

2.3.3 Obesity

Resistin and n-3 PUFA seem to be associated with the risk of obesity, as a chronic low-grade inflammation. A study among 27 men and women with body mass index (BMI) 21.7 ± 0.4 kg/m², and 50 obese with BMI 49.8 ± 1.5 kg/m² showed that obese subjects had higher resistin concentration as compared to lean subjects (Degawa-Yamauchi et al. 2003). A number of animal studies have shown that higher intake of n-3 PUFA can protect against the development of obesity by the reduction of body fat accumulation (Buckley & Howe 2009). It has been pointed out that n-3
PUFA may be a protective factor for obesity by its impact on determinants of obesity. For example, plasma concentration of n-3 PUFA was inversely associated with BMI, waist circumference (WC) and hip circumference (HC) (Micallef et al. 2009).

The correlation between resistin concentration and obesity was not supported by all studies. For example, Regarding to the cross-sectional study in 123 middle-aged women and 120 healthy young subjects, resistin concentration was not correlated with BMI, WHR, fat mass and insulin resistance. Also resistin concentrations were non different in diabetics and not diabetics (Lee et al. 2003). This finding was supported by another study among 177 non-diabetic subjects (75 male, 102 female; age 32-75 years). In this study, resistin was only associated with body fat, whereas no associations have been found between resistin concentration and BMI and waist circumference (Utzschneider et al. 2005)

2.3.4 Inflammation

Inflammation has been known as a risk factor for the beginning and progression of chronic diseases (Hansson et al. 2006). Resistin concentration and intake of n-3 PUFA are two factors that may be involved in the inflammation process. Resistin is known as a pro-inflammatory cytokine and structurally is similar to proteins, which are directly related to inflammation process (Holcomb et al. 2000, Steppan et al. 2001). Substantial evidence from epidemiological studies indicates that serum resistin concentration is directly associated with the some inflammatory markers, such as tumor necrosis factor (TNF), interleukin-6 (IL-6) and C-reactive protein (CRP) (Bo et al. 2005, Al-Daghri et al. 2005, Shetty et al. 2004, Stejskal et al. 2003).

It has been shown that serum resistin concentration was directly related to CRP concentration among 77 diabetics or participants who were at high risk to develop diabetes (Shetty et al. 2004). This association remained significant after sex and BMI adjustment. Direct association between resistin concentration and CRP has been supported in another prospective study among 213 obese participants (mean age 45.1 ± 16.7 years, and mean BMI 35.6 ± 5.7) (De Luis et al. 2009). Serum resistin level was directly correlated with CRP among 300 obese and non-obese men (Bo et al. 2005). It has been reported that resistin concentration enhances the synthesis and secretion of TNF as a pro-inflammatory cytokines (Bokarewa et al. 2005, Silswal et al. 2005).

Findings about the association between the n-3 PUFA and inflammation markers are inconsistent. In the KIHD, there was an inverse association between the serum n-3 PUFA and serum CRP concentration among 1395 middle-aged and older men (Reinders et al. 2012). Regarding to the
some observational studies findings, higher fish consumption was associated with lower CRP concentration (He et al. 2009, Kalogeropoulos et al. 2010, Kalupahana et al. 2011).

In contrast, another study could not find any association between habitual dietary intake of n-3 PUFA and CRP concentration among 859 healthy men and women (Pischon et al. 2003). Furthermore, finding from a placebo-controlled randomized trial showed that fish oil supplementation could not alter CRP concentration among healthy population with mild hypertriglyceridemia (Skulas-Ray et al. 2011).

The anti-inflammatory properties of n-3 PUFAs can be partially explained by their impact on the secretion of proinflammatory mediators (Calder 2006, Tai & Ding 2010), inflammatory eicosanoids, cytokines, and reactive oxygen species and the expression of adhesion molecules (Calder 2006, Tai & Ding 2010) and reduction of macrophage migration into adipose tissue (He et al. 2009, Kalogeropoulos et al. 2010, Kalupahana et al. 2011). For example, it has been reported that intake of n-3 PUFA, particularly EPA and DHA, are inversely associated with the production of some pro-inflammatory cytokines like TNF and IL-6 in the monocytes and endothelial cells (Babcock et al. 2002, Khalfoun et al. 1997, Lo et al. 1999, Novak et al. 2003).

2.4 Potential mechanism of the association between EPA and DHA and adipokines

The pathophysiological mechanisms of serum n-3 PUFAs and adipokines concentration have not been fully explored. However, it seems that the secretion and expression of adipokines may be influenced by intake of n-3 PUFA, particularly EPA and DHA. This role can be partially explained by the relationship between EPA, DHA and peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ is a group of the nuclear receptors, which are mainly expressed in adipose tissue, colon and macrophages and can regulate fatty acid storage, regulation of cellular differentiation and energy metabolism (Fajas et al. 1997, Houseknecht et al. 1998).

Resistin gene expression is affected by the expression of PPARγ (Way et al. 2001, Steppan et al. 2001, Patel et al. 2003). It has been suggested that the activation of PPARγ in fat can be affected by EPA and DHA (Han et al. 2014) and this association can have indirect impact on the secretion of the adipokines (Neschen et al. 2006). However, more evaluations are needed to confirm this possible mechanism.

Moreover, it has been reported the resistin expression is strongly directly associated with production of some proinflammatory cytokines such as tumor necrosis factor, interleukin-1, and interleukin-6
in peripheral blood mononuclear cells (PBMCs) (Anderson et al. 2007, Bokarewa et al. 2005). Since, N-3 PUFA may reduce production of these cytokines (Calder 2006), n-3 PUFA may play an indirect role in the secretion and expression of resistin.

Noteworthy that higher resistin expression in macrophages can decrease production of adenosine monophosphate-activated protein kinase and it leads to reduction in fatty acid uptake and metabolism in skeletal muscle, which can be another reason for the inverse association between the resistin concentration and serum n-3 PUFA concentration (Palanivel & Sweeney 2005).

2.5 Previous studies

There are few studies that evaluated the association between the n-3 PUFA and resistin concentration and expression. It has been suggested that plasma EPA and arachidonic acid may lead to a small reduction in the expression of resistin mRNA in murine adipocytes (Haugen et al. 2005). Moreover, in non-alcoholic steatohepatitis rats, resistin concentration was decreased by 8 weeks high-fat diet with n-3 PUFA (Ye Wang 2014).

In population-based studies, a randomized control trial among 38 patients with acute myocardial infraction (AMI) indicated that n-3 PUFA therapy slightly increased serum resistin concentration in the first month after AMI (Mizia-Stec et al. 2011). In contrast, regarding to the two randomized – placebo control trials among participants with stable coronary artery disease (CAD) undertaking elective percutaneous coronary intervention (PCI) and women with polycystic ovary syndrome (POS), n-3 PUFA treatments did not alter resistin concentration (Mostowik et al. 2013, Oner & Muderris 2013).

2.6 Factors affecting resistin level

For evaluating the association between the serum resistin concentration and serum n-3 PUFA, it is necessary to take into account the impact on dietary and non-dietary factors on the resistin concentration. For example, it has been suggested metabolic syndrome determinants are associated with resistin concentration (Norata et al. 2007). Metabolic syndrome is cluster of metabolic disorders, which is linked to increasing incidence of heart diseases, insulin resistance and diabetes. BMI, age, gender, blood pressure, concentrations of triglyceride, low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL), CRP concentration, use of tobacco, hormonal changes and glucose level are known as the metabolic syndrome determinants. (Mottillo et al. 2010, Timar et al. 2000).
2.6.1 Age and gender

The role of age on resistin level is still unclear. However, some studies found that resistin concentration is directly associated with the age. Regarding to case-control study among 140 type II diabetic patients and 125 control subjects, plasma resistin concentration was higher among the older diabetic participants. Also resistin concentration was higher in diabetic women was higher compared to diabetic men (Gharibeh et al. 2010). This finding was supported by another study, which was conducted among healthy population (Silha et al. 2003). In contrast, another study showed that age and gender did not have effect on resistin among healthy population (Vilarrasa et al. 2005). This finding was in agreement with another study among 120 healthy Greek students (Yannakoulia et al. 2003).

2.6.2 Anthropometric measurement

It has been pointed out that resistin concentration was directly associated with the BMI among in healthy men aged 15 to 70 (Vilarrasa et al. 2005). Another study among 114 healthy Greek students showed that serum resistin concentration was inversely associated with waist to hip ratio (WHR), while, a direct association was observed between the resistin concentration and body fat mass (Yannakoulia et al. 2003). Resistin content was slightly higher in men with normal BMI as compared to overweight and obese men (Bo et al. 2005). Moreover, resistin level was directly correlated with BMI only among normal BMI participants. It has been indicated that serum resistin level was directly associated with WC in diabetic participants (Gharibeh et al. 2010). It has been reported that resistin concentration was not associated with BMI among 17 participants with mean BMI 23 and non-diabetic obese participants with mean BMI 33 (Silha et al. 2003). Another cross-sectional study also could not find any association between the BMI and expression of resistin (Savage et al. 2001).

2.6.3 Smoking and alcohol

Smoking is known as a risk factor for several health problems and it is known as an important risk factor of CVD. Few studies have investigated the impact of smoking and alcohol on the resistin concentration. It has been pointed out that smokers had higher plasma resistin level compared to non-smokers (Vilarrasa et al. 2005). In addition, it has been demonstrated that serum resistin concentration was inversely associated with moderate alcohol consumption among women (Fargnoli et al. 2008).
2.6.4 Blood pressure

Association between resistin concentration and blood pressure is still matter of debate. It has been demonstrated that resistin concentration was directly associated with the systolic blood pressure in healthy population (Norata et al. 2007). According to a prospective cohort study, which was conducted in 213 non-diabetic obese male and female subjects, serum resistin concentration was inversely associated with the systolic and diastolic blood pressure (De Luis et al. 2009). Another study among 300 healthy participants found a direct association between serum resistin concentration and diastolic blood pressure, but after age and BMI adjustment, this association did not remain (Bo et al. 2005).

2.6.5 Dietary factors

It seems that dietary factors may have impacts on the resistin concentration. For example, it has been indicated that people with high score Alternate Healthy Eating Index (AHEI) may have lower concentration of resistin (Fargnoli et al. 2008). AHEI is the way that reflects a healthier dietary pattern. It has some parameters including intake of fruit and vegetables intake, the ratio of white meat to red meat, trans fat, the ratio of polyunsaturated fat to saturated fat (P:S), intake of cereal fiber, nuts and soy, alcohol consumption, use of multivitamin. Each of them has the scores that show the quality of individual dietary pattern (Fargnoli et al. 2008). It has been reported that some parameters like fiber intake, use of multivitamin and P: S were associated with the reduction of the resistin concentration. (Fargnoli et al. 2008). In addition, a low-caloric diet based on the increase consumption of high fiber foods, vegetables and seasonal fruits combined by 60 min per day walking led to decrease in the resistin concentration in 20 obese women (BMI > 30kg/m2) (Fethi et al. 2013).

It has been shown that resistin concentration is directly related to the intake of saturated fatty acids (Cabrera de León et al. 2014). In contrast, an inverse association has been observed between the intake of monounsaturated fatty acids (MUFA) and serum resistin concentration in generally healthy population. Another study by Greco et al. (2014) showed that Mediterranean, hypocaloric diet including carbohydrates (55% of total calories), proteins (20% of total calories), and mostly mono- and polyunsaturated fats (25% of total calories) and 25-30 g/day fiber, did not change serum resistin level among obese adults with mean BMI was 37 kg/m2 (Cabrera de León et al. 2014).

Another study, which was conducted among 114 healthy Greek students showed that total energy intake or macronutrient intake did not significantly alter resistin concentration after gender and
body fat adjustments (Yannakoulia et al. 2003). Another study among healthy and normal-weight women (age 22 ± 3 years) could not find any changes in resistin concentration after 4-weeks reduced-calorie diet. However, adiponectin and leptin levels were reduced by this diet (Wolfe et al. 2004). Noteworthy that some observational studies indicated that fasting leads to reduction in resistin mRNA expression. However, after feeding, resistin mRNA expression gets back to normal level (Kim et al. 2001; Rajala et al. 2002).

### 2.6.6 Cholesterol

Resistin mRNA expression has an inverse correlation with plasma cholesterol level (Jové et al. 2003). In addition, it has been shown that serum resistin concentration was inversely associated with HDL-cholesterol in 77 diabetics or participants who were high risk of diabetes (Shetty et al. 2004). This association was supported by another study in healthy population (Norata et al. 2007). Also it has been demonstrated that there was an inverse association between resistin level and HDL in non-diabetic obese people (De Luis et al. 2009). It has been reported that resistin level was inversely correlated with ApoAI level and directly associated with ApoAI/ApoB in women (Norata et al. 2007).

### 2.6.7 Triglycerides

It has been shown that there is a highly direct association between resistin concentration and triglyceride level (Norata et al. 2007). Regarding to a study by (Haugen et al. 2005), which was conducted among 54 adolescents, serum resistin concentration was directly correlated with the triglyceride concentration after six-month lifestyle intervention. Moreover, a direct association has been observed between the resistin concentration and triglyceride level among non-diabetic obese subjects (De Luis et al. 2009). Another study, which was conducted among 300 adult men, indicated that plasma resistin level was directly associated with triglyceride level among normal BMI participants. However, this association was strongly dependent to the glucose level (Bo et al. 2005).

### 2.6.8 Other factors

There are other factors, which affect resistin level but number of studies showing association between these factors and resistin level is limited. For example, it has been reported that resistin and leptin concentrations are related to each other (Silha et al. 2003). Moreover, serum resistin
concentration is strongly associated with plasma urea, insulin and creatinine in diabetic people (Gharibeh et al. 2010).

2.7 Therapeutic consideration
Since higher resistin concentration is associated with the risk of chronic diseases including CVD and T2D, several therapeutic approaches have been investigated the possibility of decreasing resistin concentration and resistin gene expression. Use of statins drugs including atorvastatin and simvastatin was one of the most effective approaches. It has been found that resistin concentration and resistin mRNA expression may be reduced among patients with T2D after atorvastatin treatment (Ichida et al. 2006). It has been also found that atorvastatin treatment was inversely associated with the resistin expression by the impact of this treatment on the TNF-α (Shyu et al. 2009).

Higher folic acid intake (folic acid-fortified foods or folic acid supplementation) is another treatments, which seems to be effective in lowering resistin concentration and expression. It has been reported that daily high-dose folic acid consumption (71µ per kg body weight) decreased resistin concentration in obese diabetic mice (Seto et al. 2010).

In addition to these treatments, the beneficial impact of oleic acid, mainly from olive oil has been investigated and it seems that higher consumption of oleic acid may reduce the resistin gene expression in the isolated adipocytes (L6 muscle cells) and enhance the impact of glucose-lowering therapies and rosiglitazone, which seems to be inversely associated with lower risk of hyperresistinemia (Rea & Donnelly 2006). However, the evaluation of the effect of high-dose oleic acid consumption on the resistin concentration among humans are still ongoing and future studies are needed to confirm this beneficial treatment.
3  AIM OF STUDY

Tackling chronic diseases like CVD and T2D is integrated with recognition are factors, which are associated with the risk of these chronic diseases. Intake of n-3 PUFAs, as an important dietary component and serum resistin concentration are two factors, which seem to be associated with the risk and incidence of chronic diseases. However, there is no information about possible association between them in generally healthy population.

The primary objective of the present study was to find out the association between n-3 PUFA and serum resistin concentration among middle-aged and older participants from KIHD cohort.
4 MATERIALS AND METHODS

4.1 Study design and participants

The participants of this study were drawn from people who participated in Kuopio Ischemic Heart Disease Risk Factor Study (KIHD). The primary goal of KIHD study was to investigate risk factors for CVD, atherosclerosis, and other health related issues among randomly selected men living in Eastern Finland (Salonen 1988). The baseline examination was accomplished between March 1984 and December 1989, consisted of 3235 men who were 42, 48, 54, or 60 years old. Out of total population, 2682 men (82.9% eligible) was taken into the study population. 4-Year follow-up examinations were carried out between March 1991 and December 1993 and 1038 men (88% eligible) were participated. Next round was an 11- year follow-up examinations (1998-2001), which included 854 men from second cohort and 920 new women (1774 Participants). This stage was followed by 20- year follow-up examinations, which were conducted between 2005 and 2008 among 1860 participants (1230 men and 630 women, 80.1%). The KIHD protocol was approved by the Research Ethics Committees of the University of Kuopio. All the subjects signed a written informed consent.

For the current analysis between serum long chain n-3 and serum resistin in healthy population, we used the data from the 11-year examination round. We excluded from the analysis subjects with CVD and T2D. These exclusion left 892 subjects (467 females and 425 males).

4.2 Measurements

Subjects gave blood specimens between 8:00 and 10:00 AM on Tuesday, Wednesday, or Thursday after having abstained from ingesting alcohol for 3 days, smoking for 12 hours, and eating for 12 hours. After the subject had rested in the supine position for 30 minutes, blood was drawn with Terumo Venoject VT-100PZ vacuum (Terumo Corp., Tokyo). No tourniquet was used (Salonen et al. 1992).

The number of cigarettes, cigars, and of tobacco currently smoked daily, the duration of regular smoking in years, history of myocardial infarction, angina pectoris and other ischemic heart disease, the presence of hypertension, and current antihypertensive medication were recorded using a self-administered questionnaire, which was checked by an interviewer. Reinter views to obtain medical history were conducted by a physician. The family history of CHD was defined as positive if the biological father, mother, sister, or brother of the subject had CHD history. The history of
hypertension in siblings was defined as positive if any sisters or brothers were reported to ever have had hypertension (Salonen et al. 1992).

A subject was defined as a smoker if he had ever smoked on a regular basis and had smoked cigarettes, cigars, or a pipe within the past 30 days. The lifelong exposure to smoking (cigarette pack-years) was estimated as the product of years smoked and the number of tobacco products smoked daily at the time of examination. Years smoked were defined as the sum of years of smoking regardless of when smoking had started, whether the subject had stopped smoking, or whether it had occurred continuously or during several periods. The consumption of alcohol in the previous 12 months was assessed with the Nordic Alcohol Consumption Inventory, which contains 15 items (Salonen et al. 1992).

Resting blood pressure was measured between 8:00 and 10:00AM on the examination day by one nurse with a random-zero mercury sphygmomanometer. The measuring protocol included, after a supine rest of minutes, three measurements in supine, one in standing, and two in sitting position with 5-minutes intervals. The mean of all six systolic pressure values was used in the present analyses as the systolic blood pressure and the mean of all six diastolic measurements as diastolic blood pressure (Salonen et al. 1992).

Both at baseline and at the 11-years examinations, plasma glucose was measured by using a glucose dehydrogenase method after precipitation of proteins by trichloroacetic acid. The serum samples for insulin determination were stored frozen at –80C. Serum insulin was determined with a Novo Biolabs radioimmunoassay kit (Novo Nordisk). Serum CRP was measured with an immunometric assay (Immulite High Sensitivity CRP Assay; Diagnostic Products Corporation).

4.2.1 Assessment of dietary intake

The consumption of foods at baseline was assessed with a guided food record of 4 consecutive days, one of which was a weekend day, by household measures. A picture book of common foods and dishes was used to help in the estimation of portion sizes and contained 126 of the most common foods and drinks consumed in Finland during the 1980s. For each food item, the participant could choose from 3 to 5 commonly used portion sizes or describe the portion size in relation to those shown in the book. To further improve accuracy, instructions were given and completed food records were checked by a nutritionist together with the participant. Nutrient intakes were estimated by using NUTRICA 2.5 software (Social Insurance Institution). The
software’s databank is mainly based on Finnish values of nutrient composition of foods. (Voutilainen et al. 2001).

4.2.2 Measurement of PUFA

Serum esterified and non-esterified fatty acids were determined in one gas chromatographic run without pre-separation (Laaksonen et al. 2002). Serum fatty acids were extracted with chloroform-methanol. Chloroform phase was evaporated and treated with sodium methoxide, which methylated esterified fatty acids. Quantification was performed with the reference standard purchased from Nu-check Prep Inc. (MN, USA). Each analyte had individual reference standard and recovery of analytes was confirmed with an internal standard eicosan (arachidonic acid C_{20}H_{40}O_{2}). Fatty acids were chromatographed in an NB-351 capacity column (HNU-Nordion, Helsinki, Finland) by a Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard Company, Avondale, PA, USA) with a flame ionization detector. The coefficient of variation of major esterified fatty acids and non-esterified fatty acids for repeated measurements were ~5% and ~15% respectively. The esterified fatty acids concentration were adjusted for serum low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride concentration an account of the fact that relative degree of saturation of fatty acids varies amid esterified fatty acids type (i.e., cholesterol esters, phospholipid and triglycerides). No adjustment was performed for non-esterified fatty acids. For the serum total long-chain omega-3 PUFA, we used the sum of eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acids (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:5n-3).

4.2.3 Serum resistin

Serum resistin levels were measured using enzyme-linked immunosorbent assay (ELISA) with a commercial kit (Phoenix Pharmaceuticals Inc., USA). The lower detection limit of the assay was 0.0625 ng/ml. Intra-assay coefficients of variation for the assay was 3%, while inter-assay was 10%.

4.2.4 Statistical analysis

All statistical analysis was applied using SPSS software version 21 for windows (SPSS Inc., Chicago, IL, USA). The univariate associations between the serum EPA+DPA+DHA concentration and demographic, lifestyle and clinical characteristics at baseline were assessed by means and linear regression for continuous variables and chi^2-test for categorical variables. We used linear regression models to determine the association between serum n-3 PUFAs and serum resistin level and
analysis of covariates to estimate the average resistin level in quartiles of the serum long-chain n-3 PUFA. Correlations were assessed by the Spearman’s correlation coefficients.

Two models were adjusted for possible confounders which were associated with resistin level in previous studies or association with exposures or outcomes in the present analysis. The model 1 was adjusted for age, gender and examination year. Model 2 was a multivariate-adjusted model including model 1 variables and leisure time physical activity (kcal/day), BMI (kg/m²), alcohol intake(g/week), smoking (cig packs/day), serum TG (mmol/l), systolic and diastolic blood pressure (mm Hg), serum LDL(mmol/l), serum HDL(mmol/l), serum CRP (mg/l), SAFA (energy %) and MUFA (energy%). Tests for linear trend for each model were conducted by using median values for each category of variable. All P-values were two-tailed (α = 0.05). Cohort mean was used to replace missing values in covariates (<0.5%).
5 RESULTS

5.1.1 Baseline characteristics

Baseline characteristic of participants are presented in Table 1. The mean age was 61.7 years (s.d. 6.4). The mean serum concentration as a percentage of all serum fatty acids was 2.78 % for DHA (s.d. 0.90), which was highest serum concentration among all long chain n-3 PUFAs, followed by EPA and DPA that were 1.66% (s.d. 0.92) and 0.78% (s.d. 0.16) respectively. The mean fish intake was 80.6 g/d (s.d. 123.71) at the baseline. Subjects with higher concentration of total long chain n-3 PUFA (Q4) were more likely to have higher education, income and leisure time physical activity, serum HDL-c, protein and vegetables intake, and more likely to be current smokers as compared to subjects with lower serum total long chain n-3 concentration. Furthermore, they had lower waist to hip ratio, serum triglyceride and total cholesterol, and intake of carbohydrates and saturated fatty acids (Table 1). Based on the gender, men had higher concentration of TG, LDL, higher intake of fish and total energy and lower LDL concentration as compared to the women (Table 2).

5.1.2 Serum long chain n-3 PUFA and serum resistin

The mean concentration of serum resistin was 1.92 ng/ml (s.d. 0.67 ng/ml). Results of the Model 1 adjusted for age, gender and examination year showed that serum total long chain n-3 PUFA (EPA+DPA+DHA) was inversely associated with serum resistin (Table 3). Among individual fatty acids, serum DHA had strongest inverse association with serum resistin level (Table 3). Furthermore, in the multivariate-adjusted model, the association between serum DHA and serum total long chain n-3 PUFA and serum resistin remained statistically significant, while and serum DPA and EPA were not associated with serum resistin anymore (Table 3). We could not find any differences between genders (P for interaction: 0.99)
Table 1. Baseline characteristics according to serum total n-3 PUFAs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Q1 (3.47)</th>
<th>Q2 (4.40)</th>
<th>Q3 (5.44)</th>
<th>Q4 (7.23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>223</td>
<td>223</td>
<td>223</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>61.35(6.52)</td>
<td>61.89(6.65)</td>
<td>61.48(6.35)</td>
<td>61.96(6.26)</td>
<td>0.696</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>27.2 (4.25)</td>
<td>27.3 (4.01)</td>
<td>27.1 (3.85)</td>
<td>26.8 (4.08)</td>
<td>0.461</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>25.3%</td>
<td>10.5%</td>
<td>13.4%</td>
<td>7.8%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum EPA (%)</td>
<td>0.88 (0.23)</td>
<td>1.26 (0.23)</td>
<td>1.66 (0.31)</td>
<td>2.82 (1.01)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum DPA (%)</td>
<td>0.64 (0.13)</td>
<td>0.75 (0.13)</td>
<td>0.81 (0.13)</td>
<td>0.90 (0.15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum DHA (%)</td>
<td>1.83 (0.37)</td>
<td>2.39 (0.30)</td>
<td>2.95 (0.35)</td>
<td>3.95 (0.67)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum ALA (%)</td>
<td>0.98 (0.30)</td>
<td>0.96 (0.30)</td>
<td>0.92 (0.26)</td>
<td>0.91 (0.25)</td>
<td>0.039</td>
</tr>
<tr>
<td>Serum CRP (mg/l)</td>
<td>2.87 (3.93)</td>
<td>2.33 (3.42)</td>
<td>2.68 (5.11)</td>
<td>2.40 (4.17)</td>
<td>0.492</td>
</tr>
<tr>
<td>Physical activity (kcal/d)</td>
<td>170.92 (202.37)</td>
<td>190.14 (202.16)</td>
<td>196.56 (266.70)</td>
<td>199.38 (171.87)</td>
<td>0.501</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.91 (0.08)</td>
<td>0.90 (0.08)</td>
<td>0.90 (0.09)</td>
<td>0.88 (0.09)</td>
<td>0.005</td>
</tr>
<tr>
<td>Mean SBP (mm Hg)</td>
<td>137 (18)</td>
<td>137 (18)</td>
<td>135 (18)</td>
<td>134 (15)</td>
<td>0.156</td>
</tr>
<tr>
<td>Mean DBP (mm Hg)</td>
<td>82 (9)</td>
<td>82 (9)</td>
<td>82 (9)</td>
<td>81 (8)</td>
<td>0.607</td>
</tr>
<tr>
<td>Education (years)</td>
<td>9.0 (2.91)</td>
<td>9.5 (3.38)</td>
<td>10.1 (3.61)</td>
<td>10.7 (3.79)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Income (euro)</td>
<td>12,923 (6,821)</td>
<td>14,088 (9,256)</td>
<td>14,809 (10,830)</td>
<td>15,351 (9,848)</td>
<td>0.001</td>
</tr>
<tr>
<td>Blood Glucose (mmol/l)</td>
<td>4.80 (0.44)</td>
<td>4.84 (0.43)</td>
<td>4.78 (0.46)</td>
<td>4.74 (0.41)</td>
<td>0.110</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>7.35 (4.00)</td>
<td>7.83 (4.60)</td>
<td>7.20 (4.50)</td>
<td>7.72 (20.82)</td>
<td>0.920</td>
</tr>
<tr>
<td>Serum TG (mmol/l)</td>
<td>1.38 (0.79)</td>
<td>1.21 (0.58)</td>
<td>1.13 (0.53)</td>
<td>1.00 (0.46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum LDL (mmol/l)</td>
<td>3.61 (0.89)</td>
<td>3.73 (0.91)</td>
<td>3.68 (0.92)</td>
<td>3.67 (0.94)</td>
<td>0.599</td>
</tr>
<tr>
<td>Serum HDL (mmol/l)</td>
<td>1.19 (0.29)</td>
<td>1.27 (0.29)</td>
<td>1.30 (0.31)</td>
<td>1.40 (0.33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum LDL (mmol/l)</td>
<td>3.61 (0.89)</td>
<td>3.73 (0.91)</td>
<td>3.68 (0.92)</td>
<td>3.67 (0.94)</td>
<td>0.599</td>
</tr>
</tbody>
</table>
### Dietary factors

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy (Kcal/d)</td>
<td>1891 (569)</td>
<td>1834 (558)</td>
</tr>
<tr>
<td>Fat (% E)</td>
<td>34.82 (6.22)</td>
<td>34.39 (5.85)</td>
</tr>
<tr>
<td>SAFA (% E)</td>
<td>15.17 (3.43)</td>
<td>14.69 (3.33)</td>
</tr>
<tr>
<td>MUFA (% E)</td>
<td>10.52 (2.61)</td>
<td>10.52 (2.21)</td>
</tr>
<tr>
<td>PUFA (% E)</td>
<td>4.72 (1.49)</td>
<td>4.89 (1.35)</td>
</tr>
<tr>
<td>Trans fatty acids (% E)</td>
<td>0.60 (0.18)</td>
<td>0.59 (0.19)</td>
</tr>
<tr>
<td>Carbohydrate (% E)</td>
<td>44.97 (7.10)</td>
<td>47.69 (6.33)</td>
</tr>
<tr>
<td>Protein (% E)</td>
<td>16.35 (2.88)</td>
<td>16.77 (2.69)</td>
</tr>
<tr>
<td>Alcohol (% E)</td>
<td>2.53 (4.41)</td>
<td>2.28 (3.60)</td>
</tr>
</tbody>
</table>

Abbreviation: EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexanoic acid; ALA, alpha-linolenic acid

SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL, high density lipoprotein; Values are means (s.d.) or percentages. Mean and linear regression for continuous variables, chi²–test for categorical variables.
Table 2. Summary mean (SD) of the variables evaluated in this study and the results according to the gender

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total</th>
<th>Women</th>
<th>Men</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>892</td>
<td>467</td>
<td>425</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>61.67 (6.44)</td>
<td>61.84 (6.39)</td>
<td>61.48 (6.51)</td>
<td>0.501</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>27.1 (4.05)</td>
<td>27.32 (4.65)</td>
<td>26.87 (3.25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical activity (kcal/d)</td>
<td>189.16 (211.25)</td>
<td>191.88 (230.17)</td>
<td>186.18 (188.50)</td>
<td>0.533</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>1.92 (0.67)</td>
<td>1.96 (0.66)</td>
<td>1.88 (0.68)</td>
<td>0.868</td>
</tr>
<tr>
<td>Serum EPA (%)</td>
<td>1.66 (0.91)</td>
<td>1.71 (0.93)</td>
<td>1.59 (0.89)</td>
<td>0.230</td>
</tr>
<tr>
<td>Serum DPA (%)</td>
<td>0.78 (0.16)</td>
<td>0.77 (0.16)</td>
<td>0.78 (0.17)</td>
<td>0.126</td>
</tr>
<tr>
<td>Serum DHA (%)</td>
<td>2.78 (0.90)</td>
<td>2.87 (0.91)</td>
<td>2.67 (0.35)</td>
<td>0.218</td>
</tr>
<tr>
<td>EPA+ DHA + DPA (%)</td>
<td>5.21 (1.79)</td>
<td>5.36 (1.82)</td>
<td>5.05 (1.74)</td>
<td>0.267</td>
</tr>
<tr>
<td>Serum C-reactive protein (mg/l)</td>
<td>2.57 (4.20)</td>
<td>2.75 (4.55)</td>
<td>2.38 (3.77)</td>
<td>0.105</td>
</tr>
<tr>
<td>Mean systolic blood pressure (mm Hg)</td>
<td>135.9 (17.46)</td>
<td>136.9 (17.66)</td>
<td>134.9 (17.20)</td>
<td>0.489</td>
</tr>
<tr>
<td>Mean diastolic blood pressure (mm Hg)</td>
<td>82.0 (8.91)</td>
<td>81.5 (5.59)</td>
<td>82.5 (9.23)</td>
<td>0.167</td>
</tr>
<tr>
<td>Serum TG (mmol/l)</td>
<td>1.18 (0.61)</td>
<td>1.13 (0.53)</td>
<td>1.24 (0.69)</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum HDL (mmol/l)</td>
<td>1.29 (0.31)</td>
<td>1.39 (0.31)</td>
<td>1.19 (0.31)</td>
<td>0.035</td>
</tr>
<tr>
<td>Serum LDL (mmol/l)</td>
<td>3.67 (0.91)</td>
<td>3.73 (0.89)</td>
<td>3.61 (0.94)</td>
<td>0.318</td>
</tr>
<tr>
<td>Total energy (kcal/d)</td>
<td>1831 (553.18)</td>
<td>1576 (424.75)</td>
<td>2111 (525.48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat (energy %)</td>
<td>34.03 (6.11)</td>
<td>33.45 (5.93)</td>
<td>34.70 (6.25)</td>
<td>0.270</td>
</tr>
<tr>
<td>Carbohydrate (energy %)</td>
<td>47.29 (.83)</td>
<td>48.50 (6.50)</td>
<td>45.89 (6.95)</td>
<td>0.108</td>
</tr>
<tr>
<td>Protein (energy %)</td>
<td>17.36 (3.05)</td>
<td>17.49 (2.93)</td>
<td>17.22 (3.17)</td>
<td>0.270</td>
</tr>
<tr>
<td>SAFA (energy %)</td>
<td>14.42 (3.33)</td>
<td>14.21 (3.23)</td>
<td>14.66 (3.43)</td>
<td>0.742</td>
</tr>
<tr>
<td>MUFA (energy %)</td>
<td>10.44 (2.43)</td>
<td>10.17 (2.35)</td>
<td>10.73 (2.48)</td>
<td>0.789</td>
</tr>
<tr>
<td>PUFA (energy %)</td>
<td>4.87 (1.39)</td>
<td>4.80 (1.37)</td>
<td>4.95 (1.41)</td>
<td>0.343</td>
</tr>
<tr>
<td>Fish intake (g/d)</td>
<td>80.64 (123.71)</td>
<td>69.84 (113.82)</td>
<td>92.51 (129.29)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

LDL, low density lipoprotein; SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids. Values are means (s.d.) or percentages.
### Table 3. Concentration of serum resistin in serum n-3 polyunsaturated fatty acid quartiles

<table>
<thead>
<tr>
<th>Variables</th>
<th>Serum total long chain n-3 polyunsaturated fatty acids quartile</th>
<th>( r )</th>
<th>( \beta )</th>
<th>( \text{P for trend} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (n =223)</td>
<td>2 (n =223)</td>
<td>3 (n =223)</td>
<td>4 (n =223)</td>
</tr>
<tr>
<td><strong>EPA+DPA+DHA</strong></td>
<td><strong>-0.12</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>- 0.15</td>
<td>2.03 (1.94-2.11)</td>
<td>1.96 (1.88-2.05)</td>
<td>1.86 (1.78-1.95)</td>
</tr>
<tr>
<td>Model 2</td>
<td>- 0.12</td>
<td>1.99 (1.90-2.09)</td>
<td>1.97 (1.88-2.05)</td>
<td>1.87 (1.78-1.96)</td>
</tr>
<tr>
<td><strong>EPA</strong></td>
<td><strong>-0.10</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>- 0.13</td>
<td>1.99 (1.90-2.08)</td>
<td>1.97 (1.88-2.06)</td>
<td>1.85 (1.77-1.94)</td>
</tr>
<tr>
<td>Model 2</td>
<td>- 0.09</td>
<td>1.96 (1.86-2.05)</td>
<td>1.96 (1.87-2.05)</td>
<td>1.87 (1.78-1.96)</td>
</tr>
<tr>
<td><strong>DPA</strong></td>
<td><strong>-0.09</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>- 0.10</td>
<td>1.98 (1.88-2.06)</td>
<td>2.01 (1.92-2.10)</td>
<td>1.87 (1.79-1.96)</td>
</tr>
<tr>
<td>Model 2</td>
<td>- 0.07</td>
<td>1.94 (1.85-2.03)</td>
<td>2.00 (1.91-2.09)</td>
<td>1.89 (1.80-1.98)</td>
</tr>
<tr>
<td><strong>DHA</strong></td>
<td><strong>-0.14</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Model 1</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>- 0.17</td>
<td>2.01 (1.92-2.10)</td>
<td>1.96 (1.87-2.04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 0.14</td>
<td>1.98 (1.89-2.07)</td>
<td>1.96 (1.87-2.05)</td>
</tr>
</tbody>
</table>

Abbreviation: EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexanoic acid;

Model 1 adjusted for age, gender and examination year.

Model 2 adjusted for Model 1 and physical activity, BMI, alcohol intake per week, smoking, serum TG, systolic and diastolic blood pressure, serum LDL,
6 DISCUSSION

In this cross-sectional study in a healthy middle-aged population, we found that serum total long chain n-3 PUFA (EPA+DHA+DPA), DHA and DPA concentration are inversely associated with serum resistin concentration when adjusted for age, gender and year of examination. Only DHA remained significantly associated with serum resistin level after additional adjustment for all potential confounders. In contrast, serum concentration of EPA was not associated with serum resistin concentration in our study. This suggests that fish or fish oil consumption may lead to lower serum resistin concentration; notwithstanding, the role of potential confounders cannot be wholly excluded.

Resistin is a cysteine-rich adipose-derived peptide hormone, which is originally named for its resistance to insulin (Steppan et al. 2001). It has been suggested that significant level of resistin expression predominantly occurs in macrophages, PBMCs, bone marrow, spleen and embedded in in adipose tissue (Fain et al. 2003, Janke et al. 2002, Patel et al. 2003, Xu et al. 2006) and mature adipocytes (Degawa-Yamauchi et al. 2003). Resistin is known as a pro-inflammatory cytokine that seemed to be a link between obesity and T2D (Holcomb et al. 2000, Steppan et al. 2001). Also resistin may increase the risk of arteriosclerosis by impairment of glucose and lipid metabolism (Wang et al. 2009).

EPA and DHA are derived mainly from seafood and fish oils (Saldeen & Saldeen 2006). ALA can be converted to EPA and DHA in human body in limited amounts. Since this conversion cannot meet the EPA and DHA demand, the majority of DHA and EPA are obtained from the diet (Burdge & Calder 2005, Barceló-Coblijn & Murphy 2009).

DPA is an EPA metabolite and majority of DPA circulating is from the endogenous metabolism rather than directly from the diet (for example, fish contains limited amount of DPA) (Kaur et al. 2011). Moreover, the correlation between fish consumption and circulating DPA is much weaker than the correlation between fish consumption and DHA and EPA (von Schacky & Weber 1985, Arterburn et al. 2006). Hence, serum EPA and especially DHA are the better markers for evaluation fish and fish oil consumption than DPA (Virtanen et al. 2009).

Resistin concentration and n-3 PUFA are both integrated with the health issues such as heart disease, insulin resistance and diabetes, obesity and inflammation, which suggest that there may be an association between them.
Although some animal and population-based studies experimental studies have evaluated the impact of long chain n-3 PUFA on resistin concentration and resistin expression, to the best of our knowledge, this is the first study evaluated association of serum n-3 PUFA concentration with serum resistin concentration in a healthy population. It has been suggested that EPA may promote a small reduction in expression of resistin mRNA in murine adipocytes (Haugen et al. 2005). Moreover, in rat model of non-alcoholic steatohepatitis resistin level was decreased by 8 weeks high fat diet with n-3 PUFA (Ye Wang 2014).

In population-based studies, in a randomized control trial among 38 patients with AMI and successful coronary stent implantation, results indicated that n-3 PUFA therapy raised the resistin concentration in the first month after AMI (Mizia-Stec et al. 2011). However, according to two randomized placebo control led trials among participants with stable coronary artery disease (CAD) undertaking elective percutaneous coronary intervention (PCI) and women with polycystic ovary syndrome, n-3 PUFA treatments did not alter resistin level (Mostowik et al. 2013, Oner & Muderris 2013).

The possible mechanism underlying the impact of serum n-3 PUFA on the serum resistin concentration can be partially explained by the relationship of EPA, DHA and resistin expression with PPARγ. PPARγ is a group of nuclear receptors mainly expressed in adipose tissue, colon and macrophages and can regulate fatty acid storage, regulation of cellular differentiation and energy metabolism (Fajas et al. 1997, Houseknecht et al. 1998).

It has been suggested that activation of PPARγ might be affected by serum EPA and DHA concentration (Han et al. 2014) and PPARγ may influence the secretion of adipokines (Neschen et al. 2006). It has been demonstrated that resistin gene expression was enhanced by overexpression of PPARγ (Way et al. 2001, Steppan et al. 2001, Patel et al. 2003).

It has been reported that there was a strong direct association between the resistin expression and some proinflammatory cytokines such as tumor necrosis factor, interleukin-1, and interleukin-6 in PBMCs (Savage et al. 2001, Anderson et al. 2007, Bokarewa et al. 2005). According to review study by (Calder 2006), higher n-3 PUFA intake could reduce production of these cytokines. So it may be another potential mechanism underlying the association between the resistin level and n-3 PUFA.
Noteworthy that higher resistin expression in macrophages can decrease production of adenosine monophosphate-activated protein kinase and it leads to reduction in fatty acid uptake and metabolism in skeletal muscle, which can be another reason for the inverse association between the resistin concentration and serum n-3 PUFA concentration (Palanivel & Sweeney 2005).

6.1 Strengths and limitations of the study

Use of serum long chain n-3 PUFA instead of dietary intake in our study increases accuracy of the study owing to the fact that serum long chain n-3 PUFA is the better marker for evaluation fish and fish oil consumption (Hodson et al. 2008). Wide examination of potential confounders and evaluation of association between serum long-chain n-3 PUFA in healthy population for the first time are the other strengths of our study. A limitation in our study was that our participants were middle-age subject so we cannot refer our result to general population and it can be one of our limitation.
7 CONCLUSIONS

To sum up, serum long chain n-3 PUFA and especially DHA, an objective biomarker of fish or fish oil consumption, were inversely associated with serum resistin concentration. As resistin is known as a potential marker for increased risk of obesity, inflammation, diabetes and heart diseases, the beneficial effect of serum long chain n-3 PUFA on these diseases can be partially explained by their impact on serum resistin concentration.
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